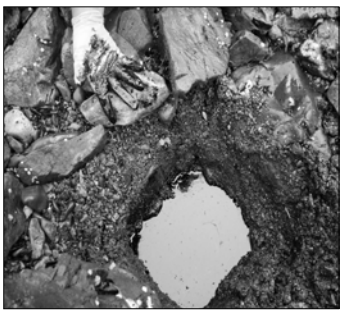
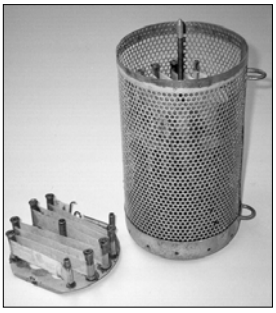
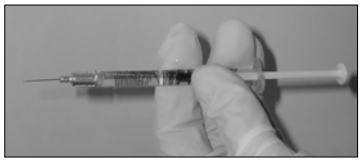
SPMDS LINK SITE-SPECIFIC CONTAMINANTS TO EFFECTS: PART I - INDUCTION OF CYP1A IN RAINBOW TROUT FROM CONTAMINANTS IN PRINCE WILLIAM SOUND, ALASKA



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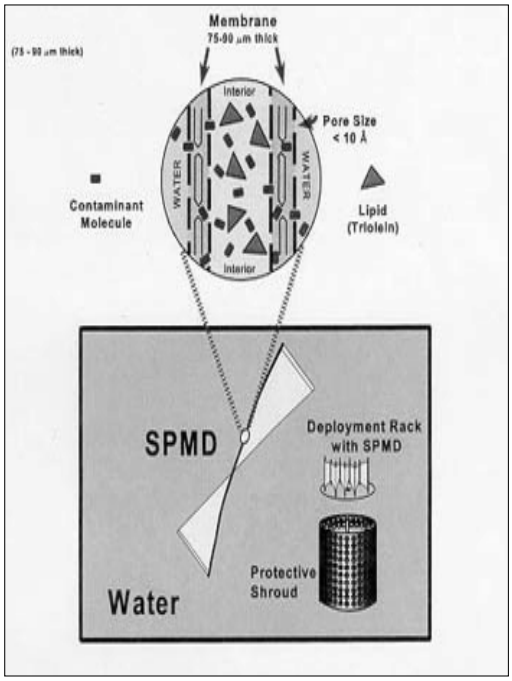
Hello, my name is Kathrine Springman, and thank you for attending this presentation. It concerns the modification of a technology and the applications of this in Prince William Sound.

SPMD Basics

- **Passive sampler**
- **Time-integrated**
- **Media-neutral (water, air, soil, sediment)**
- **Lipophilic compounds at trace levels; environmentally relevant**



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First, some introductory material.

•This study uses SPMDs, and

•SPMD stands for semi permeable membrane device.

•These are passive samplers, and they're placed in situ for a period of time, the SOP being 28 days.

•What they absorb is spread out over this time, it overcomes the snapshot effects of taking water samples and extracting them: **no pulses.**

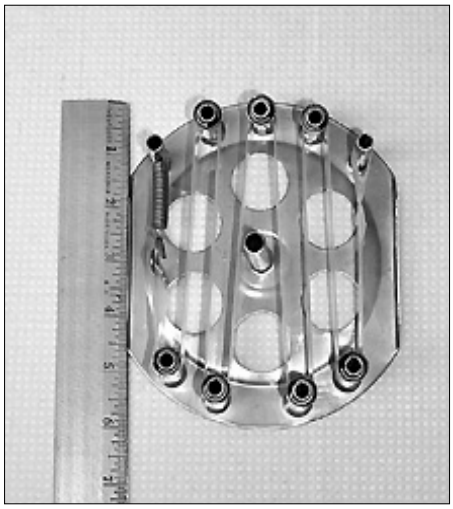
•They're a useful application of mimetic chemistry in various media, and with them, trace concentrations of lipophilic compounds, especially hydrocarbons such as PAHs, can be detected.

•SPMDs are also more user-friendly…no more liquid-liquid extractions of large quantities of water.

The graphic shows the membrane, 75-90 microns thick. It's low density polyethylene that has pores of about 10 Angstroms in size

Standard size is 91.4 centimeters long, and it contains 1 ml triolein, a neutral lipid that's associated with fish. The SPMD serves as a sink, if you will, for hydrophobic compounds.

SPMD Basics: More Advantages



- **Mimics bioconcentration process**
- **No metabolism of sorbate**
- **Not susceptible to environmental effects or pathogens**
- **Well-documented, standardized**

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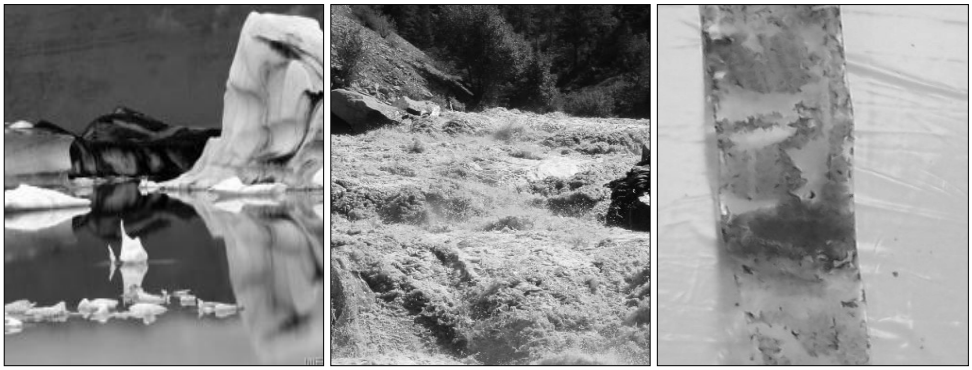
Here, you see a clean SPMD on what's called a spider. This holds the SPMD when it's deployed so as to maximize contact with the medium under study.

USGS calls the SPMD the virtual fish, as it mimics bioconcentration, and the hydrocarbons it picks up are in there when it's retrieved. They're not metabolized or depurated. The SPMD doesn't eat, or fall prey to disease, or escape. The documentation of them is extensive. They've also been patented. This patent covers the SPMD itself, and its processing which provide some valuable standardization.

Factors that affect SPMDs:

- **Temperature: less problematic than some factors**
- **Flow: greatest impact**
- **Biofouling: impedes contact, hard to control**



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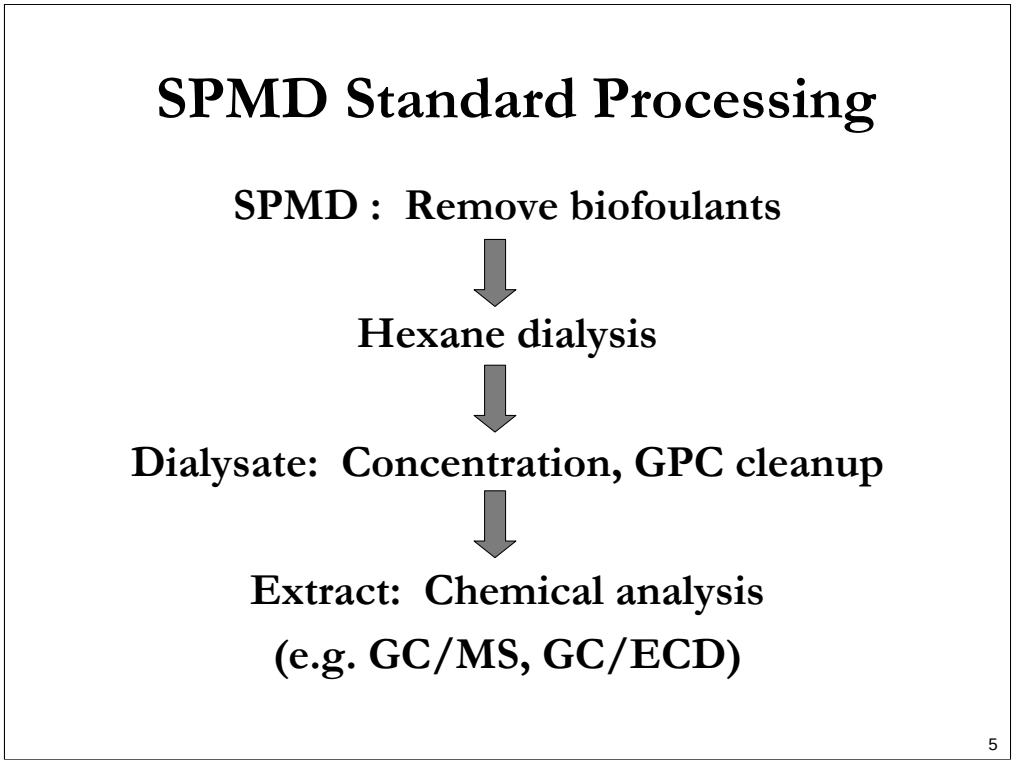
What affects uptake? Here's a few factors:

• Temperature can affect them, but there is calibration data for a wide latitude of temperatures.

• Flow does matter a great deal, as it will affect uptake

• biofouling counts, as it affects flow, and thus contact. Biofouling can be barnacles, algae, or other biota. Ask Terri Spencer at EST labs; she's got a collection of all sorts of things that have come off SPMDs.



More basics…here's the process. They're deployed for 28 days, retrieved, and then at EST labs they're cleaned off, dialyzed in analytical grade hexane in a clean room, and then the dialysate is concentrated and often the dialysate is cleaned up, making the chemical analysis easier.

SPMD Modification

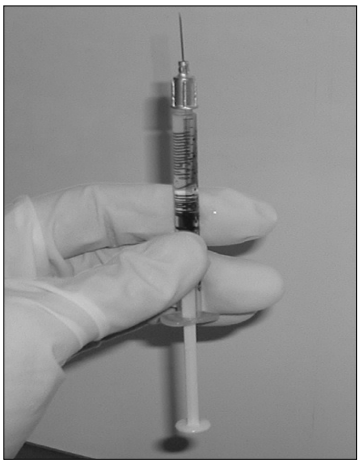
SPMD → Dialysis → Concentration → Split sample

Analysis ← Split sample

Split sample → Removal of hexane

Removal of hexane → Resuspension in solvent

Resuspension in solvent → Injection in trout fry



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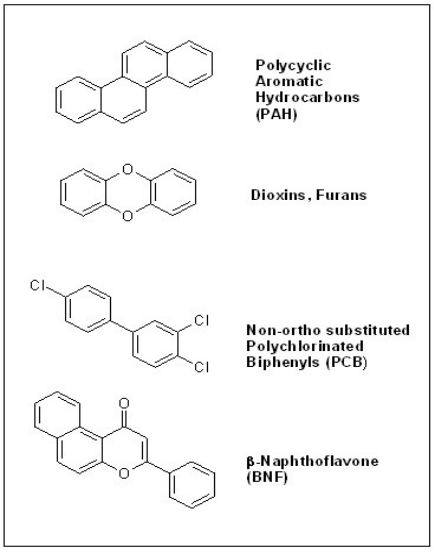
This is the modification that was designed and originally tested with spiked SPMDs. The first steps, namely dialysis and concentration, are the same. Here's the difference: after concentrating the dialysate, the sample's split. A small percentage is then sent for analysis, as that's all that's usually needed. The hexane is removed from the remainder of the sample. This is then resuspended in another solvent, and that's then injected intraperitoneally into trout fry. If you're interested in another species, that's fine. Let me know what happens.

CYP1A: Multi-purpose Enzyme

- Member of P450 enzyme family
- Substrate-inducible; low constitutive levels
- Specific for planar organics: dioxins, PAH, coplanar PCB
- Sensitive measure of oil exposure



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Research Goals

- **To determine if injecting test animals with SPMD extracts, with the modifications described, elicits a consistent biochemical response**
- **To determine if bioavailable residual oil from the *Exxon Valdez* induces CYP1A in rainbow trout fry**

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The goals of this research were simple:

• The concept of the SPMD, that of assessing bioavailable contaminants, was very appealing. With these modifications, the analytical strengths weren't lost or even affected. As the contents of the SPMD are bioavailable, this method allowed us to realistically evaluate the induction potential of the complex mixtures found at different site types in Prince William Sound. Of course, the utility of this method depended on the consistency of results obtained.

• another goal was to see if any of the residual oil from the Exxon Valdez oil spill in 1989 was bioavailable, and sufficient to induce CYP1A enzyme in rainbow trout fry. This enzyme has been the marker of petroleum exposure in many different animal species, including otter, sea ducks, and various salmonids.

Research Design

- **Deploy SPMDs, process with modifications; same for field blanks and controls**
- **Expose hatchery-raised rainbow trout fry (mean wt: 9.2 g) to SPMD extracts via i.p. injection – 50 µl per fish**
- **Allow 2 or 7 days induction**
- **Sacrifice; excise liver for ethoxyresorufin-*o*-deethylase (EROD) assay for evidence of CYP1A activity**

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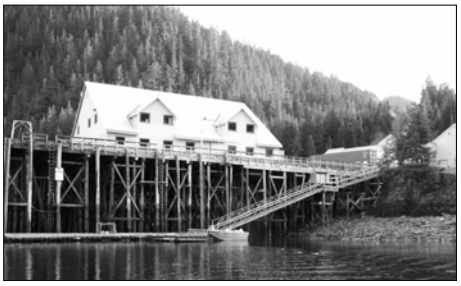
So, we deployed the SPMDs in the mid-intertidal zone in prince william sound, processed them as well as field blanks and dialysis blanks. These samples and controls were injected intraperitoneally into trout fry. After induction time of 2 or 7 days, they were sacrificed, measured, and the livers were carefully collected and assayed in the EROD assay.

Site Selection: Site Types



Above: Salmon Stream

Top: Hatchery
Bottom: Human Use

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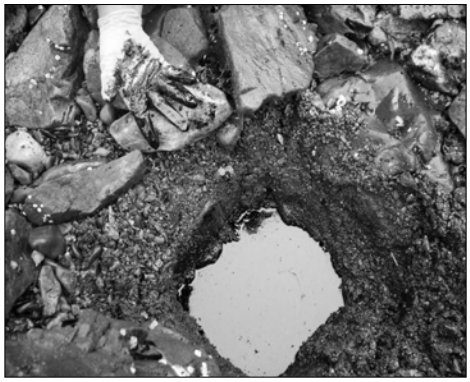
Here are the site types:

•Salmon streams, with the deployment device in the middle of the stream bed surrounded by salmon carcasses.

• hatcheries

•And there is an example of historic human use. An old cannery.

Site Selection: Site Types



Above: An oiled site on Knight Island

Below: Positive field control (hot site): Cordova Harbor

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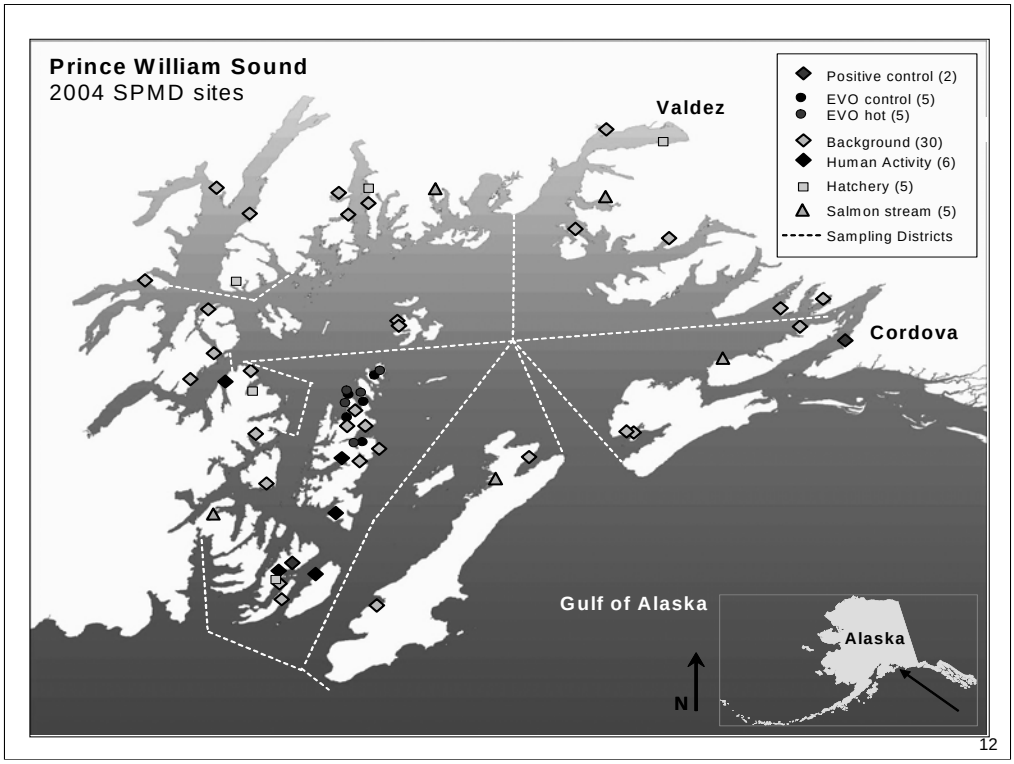
More site types

- an oiled site from knight island
- And cordova harbor. Great deal of boat traffic.



And here's where they were deployed.

•There were two positive control or hot sites, one of which was cordova harbor with all its boat traffic.

• oiled sites and local controls for those sites

• thirty background sites, although not all were used in induction, but were analyzed. One reason was to track the presence of atmospherically transported contaminants that could induce CYP1A in native biota.

• historic human activity sites such as abandoned canneries to examine their potential for inducing CYP1A in biota of the sound with their effluents

• hatcheries were included for the same reason, and for boat traffic

• and salmon streams, to check for contaminants that may have been deposited in the streams from post-spawning salmon carcasses

•You can see that we covered most of the sound for a good look at what's bioavailable.

SPMD Application: Deployment

- One deployment device = 5 SPMDs
- 28 d *in situ* (SOP)
- Placed in the mid-intertidal zone

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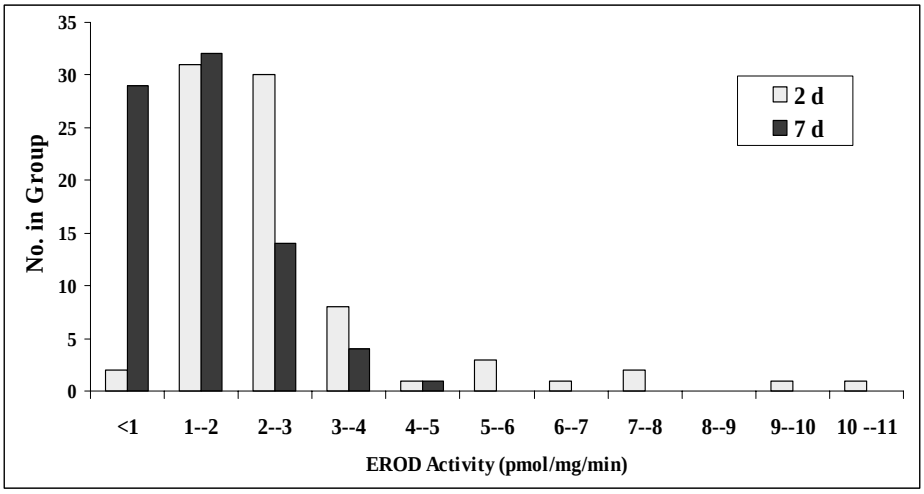
Here you see a deployment device, fully loaded with five SPMDs. These were deployed as you see in the picture here (point) for 28 days.

Results



Raw control data: pooled random, ANS control and regional control sites

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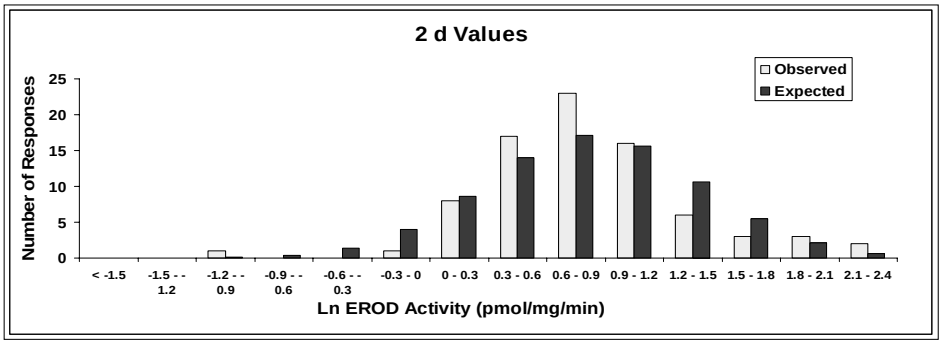
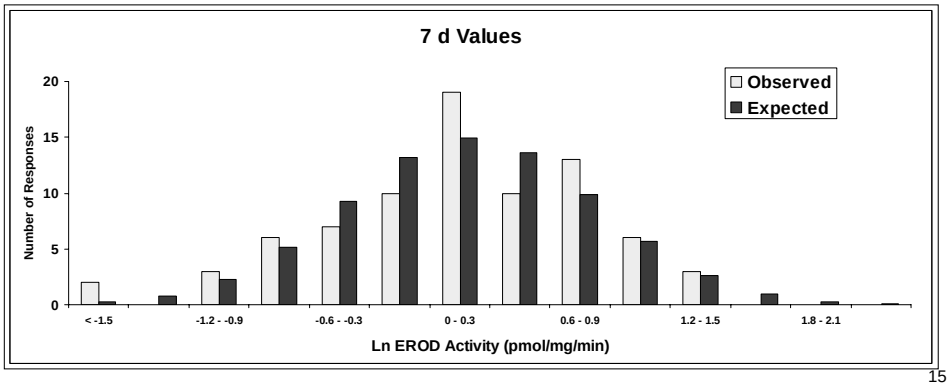

2 d Values
Observed
Expected
Number of Responses
25
20
15
10
5
0
< -1.5
-1.5 - -1.2
-1.2 - -0.9
-0.9 - -0.6
-0.6 - -0.3
-0.3 - 0
0 - 0.3
0.3 - 0.6
0.6 - 0.9
0.9 - 1.2
1.2 - 1.5
1.5 - 1.8
1.8 - 2.1
2.1 - 2.4
Ln EROD Activity (pmol/mg/min)


7 d Values
Observed
Expected
Number of Responses
20
15
10
5
0
< -1.5
-1.2 - -0.9
-0.6 - -0.3
0 - 0.3
0.6 - 0.9
1.2 - 1.5
1.8 - 2.1
Ln EROD Activity (pmol/mg/min)

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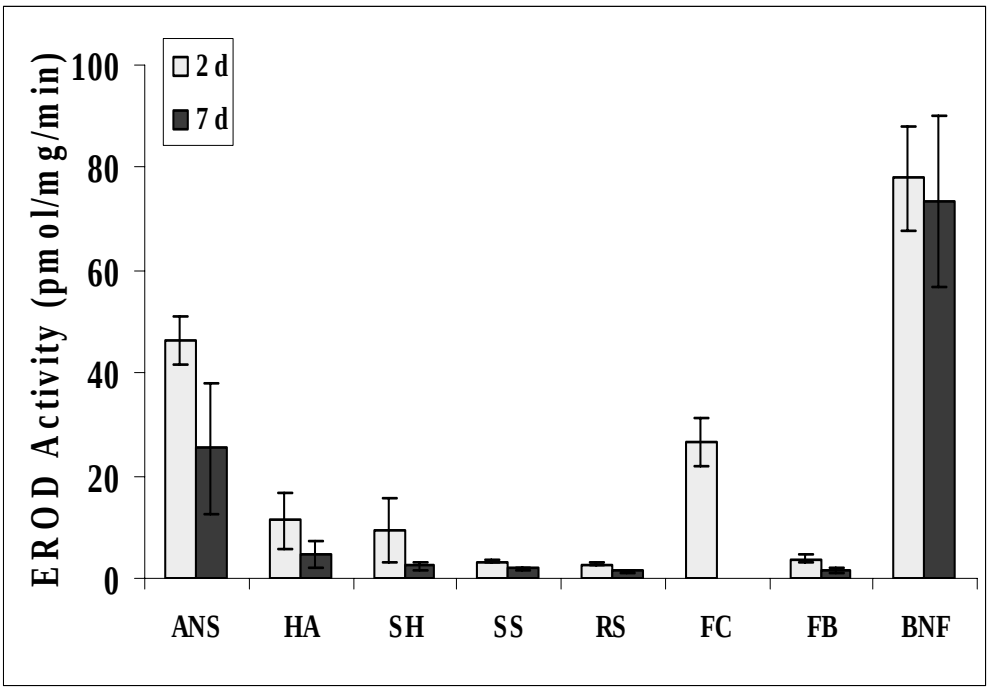
EROD Activity (pmol/mg/min)
100
80
60
40
20
0
2 d
7 d
ANS
HA
SH
SS
RS
FC
FB
BNF

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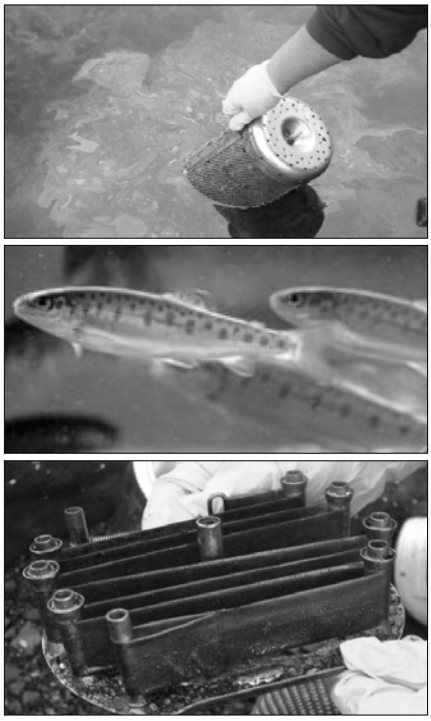
Conclusions

- **Exposing fish to SPMD contents via i.p. injection is a sensitive method for linking the bioavailable contaminants at a site to biochemical effects that result in exposed biota**
- **Residual bioavailable *Exxon Valdez* oil induces CYP1A to levels up to two orders of magnitude greater than controls**



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The results showed that the modifications used in this research are useful as the consistency was striking. The method is sensitive and reflects the response range of the test animal. Residual bioavailable Exxon Valdez oil induces CYP1A in trout fry at much higher levels than any other putative vector or contaminant source. Basically, it's not the only one there, but by far and away shows the most biological impact. Nothing comes close.

Remember the earlier picture of the SPMD on the spider? That was before exposure. On the bottom is after exposure to 15 year old oil.

Caveats and Notes

- **Dosing**
  - **Several way to calculate dosage**
- **Which comes first, the analysis or the assay?**
  - **Risk of unanticipated test organism mortalities**
- **It's in the numbers**
  - **Statistical strength can require many test organisms, which require dosing**
- **SPMD/EROD vs. field sampling**
  - **A complement, rather than replacement**

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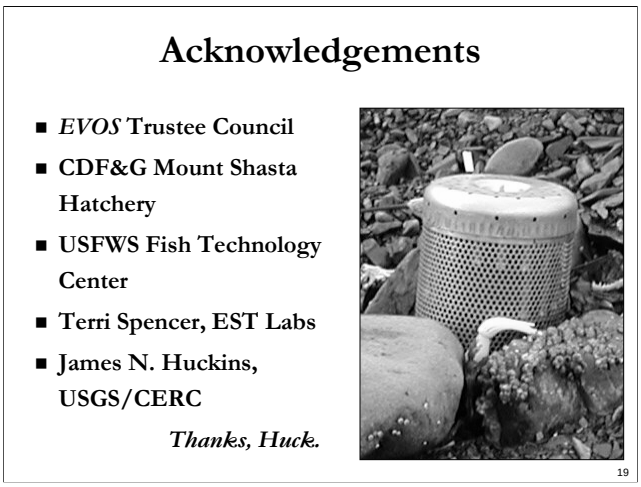

Here are the grateful acknowledgments that are the best part of any presentation, at least for the presenter.

I would like to express my gratitude to those with whom I was fortunate enough to work: Colin Kahn, Peter Hodson,and Jeep Rice, Jeff Short and Mandy Lindeberg. Thank you for providing the opportunity.

•To the EVOS trustee council, for funding this work

•My thanks to California department of fish and game for their professionalism, their assistance, and their fish. CDFG is a delight to work with.

•US fish and wildlife service, to mark maskill, and to the folks at the fish technology center in bozeman, montana. They made the pilot studies of this go smoothly

•Terri spencer, thank you. For everything.

•Finally, to james n. huckins, whose work has been dependably clear, well reasoned, and creative; this work was straightforward because of the elegant approach you and others at CERC so methodically documented. Your work will be sorely missed. Thanks huck.

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- **Contact for reprints, additional information**